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EXHIBIT "K"

HEPARAN SULFATE GLYCOSAMINOGLYCANS AS PRIMARY CELL SURFACE RECEPTORS FOR HERPES SIMPLEX VIRUS

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INTRODUCTION

The purposes of this communication are (i) to summarize the evidence that heparan sulfate moieties of proteoglycans serve as cell surface receptors for herpes simplex virus (HSV) and (ii) to describe the heparin-binding viral glycoproteins that mediate the binding of virus to cells. These topics are discussed in the context of the molecular interactions required for entry of HSV into cells and, also, the pathology of HSV infections.

HERPES SIMPLEX VIRUSES TYPES 1 AND 2: DISEASES AND BIOLOGY

The most common form of disease caused by HSV in humans is manifested as mucocutaneous lesions, which occur usually in or near the mouth (cold sores or fever blisters) or eyes (keratitis) or on genital tissues. Because the virus that causes primary lesions establishes a latent infection in the ganglia of sensory nerves and can be reactivated by appropriate stimuli, periodic recurrence of herpetic lesions is common and is one of the most troublesome aspects of infections with HSV. Although it happens less frequently, HSV can also cause life-threatening disease affecting vital organs, including encephalitis in apparently normal adults and disseminated disease in infants and immunocompromised individuals.

There are two distinct forms of HSV, designated types 1 and 2 (HSV-1 and HSV-2). The viruses isolated from cases of adult encephalitis, keratitis and facial lesions are usually HSV-1 whereas those isolated from cases of neonatal disease and genital lesions are usually HSV-2. This apparent preference of the two HSV types for different parts of the human anatomy could be due principally to the usual source of inoculum for infection at each site. Transmission of virus from one individual to another occurs most readily when there is close contact with mucosal surfaces from which virus is being shed. In addition, HSV-1 and HSV-2 may differ intrinsically in their ability to establish infection at different sites, perhaps due in part to preferential use of different cell surface receptors that are not equally represented at the different sites. Before any information was available as to the nature of HSV receptors, evidence was presented that the cell surface receptors for HSV-1 and HSV-2 are not identical (Vahlne *et al.*, 1979; Vahlne *et al.*, 1980; Addison *et al.*, 1984).

The genomes of HSV-1 and HSV-2 are closely related in that each gene of HSV-1 has an homologous counterpart in HSV-2 and the genes are arranged on the DNA in

Heparin and Related Polysaccharides

Edited by D.A. Lane *et al.*, Plenum Press, New York, 1992

the same colinear arrangement (McGeoch, 1989). Recombination readily occurs between HSV-1 and HSV-2 to yield viable progeny, implying functional as well as sequence homology of related genes. Nevertheless, divergence between homologous genes of the two types has been global and significant, such that overall identity or close similarity of DNA sequences is about at the level of 50%. Different HSV strains of the same type also exhibit genetic diversity, although to a much lesser degree. One of the questions to be raised in this communication is whether genetic variations in the viral proteins that mediate the binding of virus to cells may influence receptor recognition and tissue tropism.

Although HSV infects principally humans outside the laboratory, animals of many species can be infected experimentally. Most vertebrate cells that can be propagated in cell culture, especially adherent cells, are also susceptible to HSV infection. The extremely broad host range of this virus implies that receptors for the virus must be highly conserved in evolution and widely distributed on many cell types.

The virus particles (or virions) of HSV-1 and HSV-2 have the typical morphology of herpesviruses. The virions consist, from the inside out, of a core containing the DNA genome, an icosahedral protein shell that encloses and protects the core, a poorly defined layer of protein located between the outer surface of the protein shell and the inner surface of the limiting envelope and, finally, the outer limiting envelope itself. This envelope is a lipid bilayer containing approximately ten viral membrane glycoproteins.

Events in the replication of HSV by a cell may be summarized as follows. Specific viral membrane glycoproteins mediate the binding of HSV to the surface of a cell. Entry of HSV into the cell occurs by fusion of the viral envelope with the cell plasma membrane. Endocytosis of HSV may occur but appears to play little or no role as a pathway of entry that can lead to infection of the cell. After fusion of the virus with the cell and disassembly of the nucleocapsid (the icosahedral protein shell containing the viral genome), the viral DNA is transported to the cell nucleus where it serves as template for viral gene transcription and for genome replication. Progeny nucleocapsids are assembled in the cell nucleus and acquire an envelope by budding through virus-modified patches of the inner nuclear membrane. The progeny virions are then transported out of the cell through a secretory pathway that appears to include the Golgi apparatus.

Relevant literature on these topics has been reviewed by Corey and Spear (1988) and Roizman and Sears (1990).

CELL SURFACE RECEPTORS FOR HSV

The hypothesis that cell surface heparan sulfate serves as receptor for HSV has its origins in results reported about thirty years ago. It was shown in these early studies that heparin and other sulfated polysaccharides could inhibit HSV infection (Nahmias and Kibrick, 1964; Takemoto and Fabisch, 1964; Vaheri, 1964). Recent investigation revealed that heparin inhibits the binding of HSV (both HSV-1 and HSV-2) to cells and that virions can bind to heparin affinity columns (WuDunn and Spear, 1989). In addition, it was shown that treatment of cells with heparitinase or heparinase, but not chondroitin lyase, destroys receptors for HSV (WuDunn and Spear, 1989). Binding of HSV-1 or HSV-2 to cells treated with the former enzymes was significantly impaired and the cells were resistant to HSV infection. Control experiments showed that the treated cells remained fully susceptible to other viruses.

Cell mutants defective in various aspects of glycosaminoglycan synthesis have been used to explore further the role of heparan sulfate in HSV infection. Mutants isolated and characterized by Esko and colleagues were derived from the Chinese hamster ovary (CHO)-cell line and are described in Table 1. Studies with these cell mutants (Shieh *et al.*, 1991) revealed the following (Table 1): (i) Whereas various strains of HSV-1 and HSV-2 could bind to wild-type CHO cells and could infect the

Table 1. HSV binding to, and infection of, wild-type CHO cells and mutants

Cell line	Biochemical deficiency	GAGs produced		References on cell lines	HSV binding and infection (% of control) ^a
		Heparan sulfate	Chondroitin sulfate		
Wild-type K1	None	Yes	Yes		(100)
Mutants:					
pgsA-745	Xylosyltransferase	No	No	Esko et al. (1985)	<1
pgsB-761	Galactosyltransferase	No	No	Esko et al. (1987)	<1
pgsD-677	N-acetylglucosaminyl & glucuronosyltransferases	No	Yes ^b	Esko et al. (1988)	<1
pgsE-606	N-sulfotransferase	Yes ^c	Yes	Lidholt et al. (1991) Bame & Esko (1989) Bame et al. (1991)	30-40

^a Compared with the wild-type K1 cells, the mutant cells were impaired as indicated, both for the binding of radiolabeled HSV and for susceptibility to HSV infection, the latter as judged by quantitating the expression of an immediate-early viral protein (Shieh *et al.*, 1991).

^b Chondroitin sulfate accumulates to levels three times higher than in wild type cells.

^c The heparan sulfate produced is undersulfated by a factor of two to three.

cells with varying degrees of efficiency, all the virus strains bound very poorly if at all to, and failed to infect, the CHO mutants that are defective for heparan sulfate synthesis. (ii) The CHO mutant that is defective for heparan sulfate synthesis, but overproduces chondroitin sulfate, was no more susceptible to HSV infection than the cell mutants that produce neither heparan sulfate nor chondroitin sulfate. (iii) The CHO mutant that produces undersulfated heparan sulfate had fewer HSV receptors than did wild-type cells and was intermediate in susceptibility to HSV infection.

All these results taken together show that the binding of HSV to cells and infection of the cells requires the presence of heparan sulfate on the cell surface. Other glycosaminoglycans cannot substitute for heparan sulfate and the level of sulfation of the heparan sulfate influences the amount of virus that can bind to the cells.

Does this mean that heparan sulfate is the receptor for HSV? For other kinds of cell surface molecules, the evidence summarized above would be considered sufficient to draw this conclusion. For heparan sulfate, however, certain properties of the molecule complicate interpretation of the results. First, heparan sulfate, like other glycosaminoglycans, is highly charged and binds with varying affinities and degrees of specificity to many proteins. The fact that HSV requires heparan sulfate on the cell surface, and cannot make do with chondroitin sulfate, implies that non-specific ionic interactions alone cannot be responsible for the binding of virus to cells. An additional indication of specificity is the finding that one viral glycoprotein can be assigned primary responsibility for the binding of virus to cells and that this glycoprotein has affinity for heparin (Herold *et al.*, 1991).

Second, heparin and heparan sulfate are best known as modulators of protein-protein interactions, as is amply demonstrated by other contributions in this volume and elsewhere. For example, it was recently shown that high-affinity binding of basic fibroblast growth factor (bFGF) to cells requires the dual interaction of bFGF with cell surface heparan sulfate (or exogenous heparin) and a protein receptor for bFGF. bFGF failed to bind to an appropriate protein receptor on cells deficient in heparan sulfate or on cells deficient in the sulfation of heparan sulfate, unless exogenous heparin was added (Yayon *et al.*, 1991; Rapraeger *et al.*, 1991). We investigated the possibility that the role of cell surface heparan sulfate in HSV infection might be to facilitate binding of the virus to some other receptor, by testing whether exogenous

heparin could substitute for cell surface heparan sulfate. We found, however, that there was no enhancement in the binding of HSV to heparan sulfate-deficient cells, or in infection of the cells, over a wide range of heparin concentrations (Shieh *et al.*, 1991). Therefore, it seems that HSV requires cell-bound heparan sulfate, presumably in the form of membrane proteoglycan.

The simplest interpretation of the findings summarized here is that heparan sulfate serves as receptor for the initial binding of HSV to cells. This binding of virus to heparan sulfate could serve to initiate a cascade of interactions between components of the viral envelope and the cell surface. Because the viral envelope contains as many as ten viral glycoproteins, of which at least half play some role in virion binding and penetration, the possibility exists for several kinds of virion-cell surface interactions to occur between the initial attachment of the virus to the cell and fusion of the virion envelope with the plasma membrane. If any of the viral glycoproteins engages in interactions with receptors other than heparan sulfate, however, these interactions must usually occur secondary to the binding of virus to heparan sulfate and/or must not be of high enough affinity to enable efficient binding of virus to cells in the absence of heparan sulfate. It should be noted here that the protein receptor for bFGF plays no role in the binding of HSV to cells or in infection of cells (Shieh and Spear, 1991; Mirda *et al.*, 1991) despite previous reports suggesting this possibility (Kaner *et al.*, 1990; Baird *et al.*, 1990).

The cell surface heparan sulfate moieties required for HSV infection are presumably constituents of proteoglycans. It remains to be determined whether any heparan sulfate proteoglycan can serve as receptor or whether a particular proteoglycan is required. Specific proteoglycans may be preferred as HSV receptors due to characteristic modifications of their heparan sulfate chains. Also, HSV may bind to diverse heparan sulfate proteoglycans but be activated for penetration of the cell only after interaction with particular proteoglycans, due perhaps to interactions of the virus with molecular determinants of both heparan sulfate and the protein core or to requirement for proteoglycans that are physically linked to other cell components involved in viral penetration.

DO HSV VIRIONS CONTAIN A HEPARINASE OR HEPARITINASE?

Some viruses that use cell surface polysaccharides as receptors also encode enzymes that can hydrolyze the viral receptors. For example, the envelope of influenza virus contains two glycoproteins, designated hemagglutinin and neuraminidase. The hemagglutinin mediates the binding of virus to cells by binding to sialic acid on cell surface glycoproteins and glycolipids. The neuraminidase can cleave off the sialic acid moieties recognized by the hemagglutinin. The biological role of the neuraminidase is not fully understood. It is believed to play an important role in permitting the release of progeny virus from infected cells. It has also been suggested that the neuraminidase may be important during the initial stages of infection, to permit virus bound to non-productive receptors to be released for re-binding to other receptors. Influenza neuraminidase is the topic of a recent review (Air and Laver, 1989).

This feature of other viruses suggested the possibility that HSV might have an envelope-associated enzyme capable of cleaving the heparan sulfate chains to which virus binds. To test this possibility, both heparin and heparan sulfate were incubated with purified virions or with commercially available heparinase or heparitinase. Whereas degradation of both substrates by the appropriate enzyme was observed, no degradation of either substrate was detectable in the samples incubated with purified virions (Fig. 1). We have calculated that, if there were only one molecule of enzyme per virion with specific activity comparable to that of heparinase, enzymatic activity should have been detectable at the highest concentration of virions tested.

Although the methods used may not have permitted detection of a hydrolase that generates only large fragments of heparan sulfate, it appears that there is no heparinase-like or heparitinase-like enzyme associated with HSV virions.

Alternatively, the enzyme is latent and requires special conditions (for example, binding to cells) for activation. It should be noted that absence of this kind of enzymatic activity is not inconsistent with the biology of HSV. Progeny virions tend to remain tightly associated with the surfaces of the cells that produced them, suggesting that the virus has no specific mechanism for release.

THE HSV GLYCOPROTEINS REQUIRED FOR INFECTIVITY

Four of the ten known membrane glycoproteins encoded by HSV have been shown to participate in the binding of virus to cells or in penetration of virus into cells. This is not to imply that the other six glycoproteins are non-participants in these processes. Rather, insufficient information is available to permit judgments as to their roles in infectivity.

The HSV glycoprotein designated gC plays a principal role in the binding of virus to cells. This conclusion is based on findings that gC has affinity for heparin and that mutant virions devoid of gC are significantly impaired in ability to bind to cells (Fig. 2) (Herold *et al.*, 1991). The reduced specific infectivity of gC-negative virions (less than 1/10th that of wild-type virus) can be explained by the reduced binding of virions to cells and also by the less efficient penetration of the virus into cells. Interestingly, gC is not absolutely required for HSV infectivity. Mutant virions devoid of gC can bind to cells and initiate infection, albeit inefficiently. What are the requirements for infection of cells by gC-negative virions? We have found that the presence of cell surface heparan sulfate is required and that exogenous heparin can inhibit binding and infection, just as for wild-type virus (Herold *et al.*, 1991). Because a second HSV glycoprotein (designated gB) also binds to heparin, we suspect that gB mediates the binding of virus to cells when gC is absent.

The other three glycoproteins known to influence HSV infectivity (gB, gD and gH) appear to be essential for viral penetration into cells. Mutations in any of these

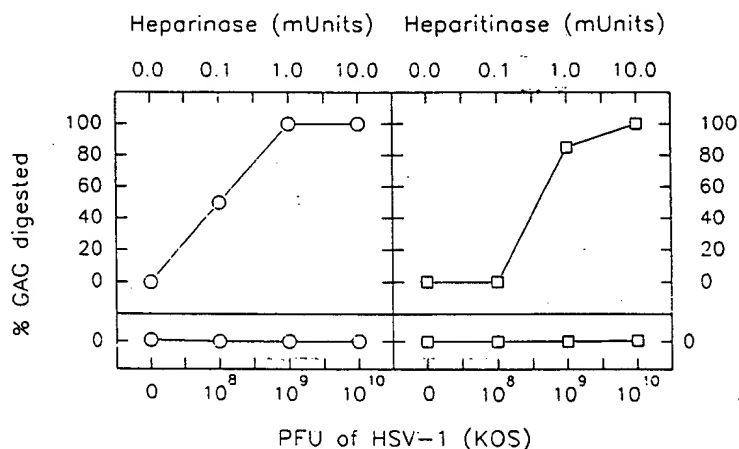


Fig. 1. Absence of heparinase-like or heparitinase-like activities on HSV-1 virions. Reaction mixtures (1 ml in 2.5 mM calcium acetate) containing heparin (circles) or heparan sulfate (squares) at 10 ng/ml and purified HSV-1(KOS) virions (lower panels) or heparinase or heparitinase (upper panels) at the concentrations indicated (units per ml) were incubated at 37°C for 1 hr. Samples of 0.1 ml were removed and added to 0.9 ml of Azure A (10 ug/ml). The metachromatic shift of the dye in response to heparin or heparan sulfate (Jaques *et al.*, 1974) was monitored at 620 nm to determine the concentrations of undigested GAG. The response of the Azure A to both GAGs was linear over the range of 0 to 1 ng. Similar results were obtained using an alternative buffer (PBS plus 1 mM CaCl₂). This assay was adapted from one described by Galliher *et al.* (1981). Unpublished results obtained by T. Koshy.

glycoproteins can reduce infectivity by several orders of magnitude (Desai *et al.*, 1988; Ligas and Johnson, 1988; Little *et al.*, 1981; Sarmiento *et al.*, 1979). Mutant virions devoid of gB or gD have been shown to bind to cells normally but to be defective in penetration (Cai *et al.*, 1988; Ligas and Johnson, 1988). Moreover, monoclonal antibodies specific for any of these three glycoproteins can block the penetration of virus into cells without impairing the binding of virus to cells (Fuller and Spear, 1987; Highlander *et al.*, 1987; Highlander *et al.*, 1988; Fuller *et al.*, 1989). Recent studies have shown that isolated truncated forms of gD can bind to cells, suggesting that gD interacts with its own, yet to be identified, cell surface receptor (Johnson *et al.*, 1990). The fact that gD-negative virions bind to cells with normal efficiency, however, indicates that gD probably does not play any role in the initial attachment of virus to cells. The fact that gB-negative virions also exhibit no obvious impairment in binding to cells (Fig. 2) (Cai *et al.*, 1988; Herold *et al.*, 1991) indicates that the heparin-binding activity of gB is not important for virus binding when gC is present.

The following sections describe more fully the available information about gB and gC, the two viral envelope glycoproteins known to have affinity for heparin (Herold *et al.*, 1991).

Glycoprotein B

Glycoprotein B is present in infected cells and virions as an oligomer, probably a homodimer or homotrimer of gB (Sarmiento and Spear, 1979; Claesson-Welsh and Spear, 1986). This oligomer is very stable, even in the presence of ionic detergent, but can be dissociated by heat. The virion-associated oligomer can be visualized by electron microscopy as a rod-like spike projecting about 14 nm from the surface of the envelope (Stannard *et al.*, 1987).

Some of the features of the gB gene product that can be deduced from nucleotide sequence analysis are summarized in Fig. 3. The N-terminal hydrophobic segment has characteristics of a cleavable signal peptide and was shown experimentally to function as such (Claesson-Welsh and Spear, 1987). Other hydrophobic segments are present

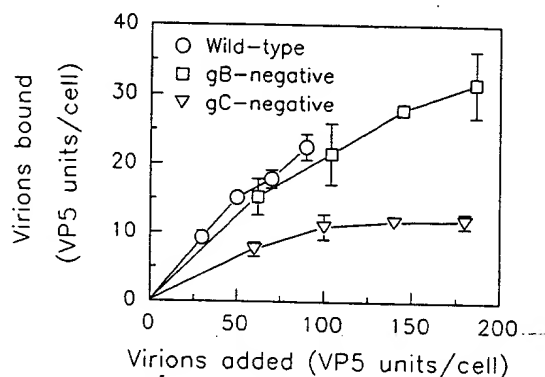


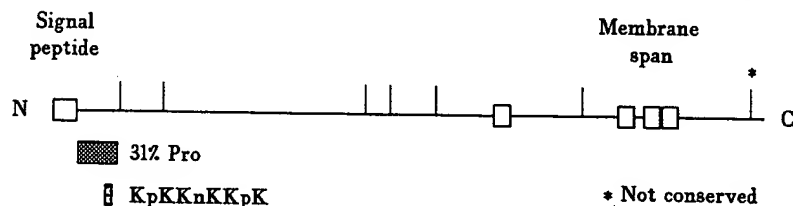
Fig. 2. Binding of wild-type and mutant HSV-1 virions to human HEP-2 cells. The wild-type virus was strain KOS and the mutants were K082 (gB-negative) and gC3 (gC-negative), both derived from strain KOS. Various concentrations of purified [35 S]methionine-labeled virions were added to the cells, which had been plated in 96-well round-bottom plates. Binding was for 2 hr at 4°C. After unbound virus was washed away, the cells were solubilized and cell-bound radioactivity was quantitated. The relative concentrations of input virions were determined by densitometry of a silver-stained SDS-PAGE gel and are expressed as VP5 units. VP5 is the highly conserved major protein of the capsid and is present in a constant number of copies per virion. The input and bound virus was determined from the known ratios of radioactivity and VP5 units for each virus preparation. Each point is the average of triplicate determinations, and the error bars represent standard deviation. Modified from Fig. 4 presented by Herold *et al.* (1991).

ward the C-terminus. It has been suggested that gB spans the membrane three times, with the three most C-terminal hydrophobic domains serving as the membrane-spanning domains (Pellett *et al.*, 1985). This is consistent with the results of experiments done to determine the orientation of the termini of the gB translation product (Claesson-Welsh and Spear, 1987). Glycoprotein B is modified by the addition of both N-linked and O-linked glycans (Johnson and Spear, 1983). The potential sites for the addition of N-linked glycans are shown in Fig. 3. Also noted in Fig. 3 is a region that has high local concentrations of basic amino acids, particularly lysine, and proline. This region, which is located at the N-terminus of the mature protein, is a good candidate for a heparin-binding domain.

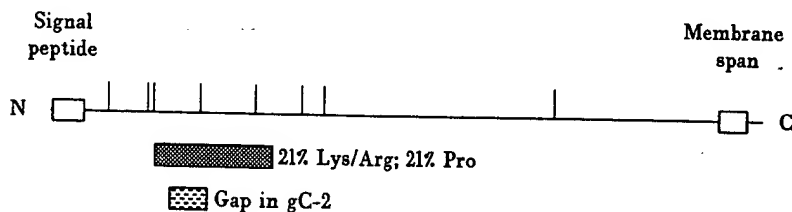
Glycoprotein C

Glycoprotein C exists in infected cells and virions probably as a monomeric protein (Sarmiento and Spear, 1979). This glycoprotein cannot readily be visualized in virions by standard techniques of electron microscopy but its presence in virions can be demonstrated by the use of monoclonal anti-gC antibodies coupled to colloidal gold. Antigenic determinants of gC extend as far as 15 to 20 nm from the virion envelope surface, suggesting that this glycoprotein is present as a very slender extended molecule (Tannard *et al.*, 1987).

Fig. 4 summarizes some of the known features of gC. Its N-terminus has properties of a cleavable signal sequence. The actual N-terminus of the mature protein has not yet been determined, however. The hydrophobic segment near the C-terminus is a membrane-spanning region. The larger N-terminal ectodomain is modified by the addition of N-linked glycans and a considerable number of O-linked glycans as well.



3. Structural features of HSV-1 gB. Hydrophobic regions that could be membrane-spanning or membrane-associated are indicated by the open boxes. One of these hydrophobic regions is a signal peptide that is cleaved off during processing of the translation product. The three hydrophobic regions near the C-terminus have been proposed to enable this protein to span the membrane three times. The vertical lines indicate the positions of potential sites for the addition of N-linked glycans (the site near the C-terminus is not conserved in HSV-2, is on the cytoplasmic side of the membrane and therefore is not likely to be modified by the addition of carbohydrate). The lysine-rich and proline-rich region that might interact with heparin is indicated. Based on several published nucleotide sequences and interpretations (Bzik *et al.*, 1984; Bzik *et al.*, 1986; Pellett *et al.*, 1985; McGeoch *et al.*, 1988).



4. Structural features of HSV-1 gC. The features indicated are as described in Fig. 3. This glycoprotein spans the membrane only once and has a very short C-terminal cytoplasmic domain. The region rich in basic amino acids and proline is indicated. The HSV-2 form of gC (gC-2) is shorter than gC-1, due to an apparent deletion of part of the domain that is rich in basic amino acids. Based on several published nucleotide sequences and interpretations (Swain *et al.*, 1985; Dowbenko and Lasky, 1984; Frink *et al.*, 1983; Draper *et al.*, 1984; McGeoch *et al.*, 1988).

(Johnson and Spear, 1983; Olofsson *et al.*, 1981; Olofsson *et al.*, 1983; Dall'Olio *et al.*, 1985). Similar to gB, gC has a region near the N-terminus that is rich in basic amino acids and proline and is a good candidate for a heparin-binding domain. The putative heparin-binding domains of gB and gC are not similar or related in actual amino acid sequence.

COMPARISONS OF HSV-1, HSV-2 AND OTHER HERPESVIRUSES

HSV-1 and HSV-2

HSV-1 and HSV-2 are biologically quite similar. From the symptoms of HSV disease in an individual patient, it is not possible to tell whether the causative virus is HSV-1 or HSV-2. Nevertheless, the two HSV types have diverged significantly, with respect to gene sequences, perhaps due in part to occupying different niches in human tissues. It is interesting to consider whether HSV-1 and HSV-2 might differ in specificity for cell surface receptors.

It seems clear that heparan sulfate moieties of cell surface proteoglycans serve as initial receptors for both HSV-1 and HSV-2. Yet there is evidence to suggest that cells can have different numbers and ratios of receptors for the two different types of HSV (Vahine *et al.*, 1980). At least two hypotheses can be proposed to reconcile these apparently contradictory findings. First, the possibility exists that HSV-1 and HSV-2 forms of the viral heparin-binding glycoproteins recognize distinct and different structural features of heparan sulfate (i.e. different receptors present on heparan sulfate chains). This implies that either gB or gC or both bind to heparan sulfate with some degree of specificity and that the HSV-1 and HSV-2 forms of gB and/or gC differ in this specificity. Second, the possibility exists that the amount of virus bound to a cell depends not only on the amount and properties of cell surface heparan sulfate but also on the number and properties of other secondary cell surface receptors. HSV-1 and HSV-2 might differ in their specificities for interaction with these putative secondary receptors. These two hypotheses are not mutually exclusive.

If the first hypothesis is correct, then the heparin-binding domains of gB and/or gC should differ in sequence for HSV-1 and HSV-2. Although the heparin-binding domains have not yet been identified, the lysine-rich regions near the N-termini of both gB and gC do exhibit considerably more divergence between HSV-1 and HSV-2 than do other regions of these proteins. The amino acid sequences of gB for strains belonging to the same type are 98-99% identical. The same is true for gC. On the other hand, there is about 85% identity of aligned sequences for the HSV-1 and HSV-2 forms of gB and about 64% identity of aligned sequences for the HSV-1 and HSV-2 forms of gC. For the N-terminal 100 amino acids of the mature glycoproteins, however, the sequence identities are only 60% and 30%, respectively. The actual amino acid sequences at the N-termini and the alignments are shown in Fig. 5. Note that alignment of the HSV-1 and HSV-2 gC sequences requires introduction of a large gap into the HSV-2 sequence right in the middle of the lysine-rich region (Figs. 4 and 5). Also, even though the four sequences available for HSV-1 forms of gB are highly conserved, almost all the sequence variation seen is at the N-terminus, as indicated in Fig. 5. These sequence comparisons are provocative and will guide efforts, at least initially, to explore the specificity and affinity of interactions between gB/gC and heparan sulfate, with attention focused on the sequence requirements of the glycoproteins as well as the heparan sulfate.

HSV and other herpesviruses

Members of the large herpesvirus family have been divided into three sub-families (alpha, beta, and gamma) on the basis of biological properties. HSV is the prototype of the alpha sub-family.

Results obtained with two other members of the alpha sub-family (pseudorabies virus [PRV] of pigs and bovine herpesvirus type 1 [BHV-1]) reveal striking similarity

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                                p l p a t K n
gB-1 (17)  apsspgtp---gvaaatqaanggpapppagapptgdpKpKKnRKpKp - 77
           ::::: ::::: ::::: ::::: ::::: ::::: :::::
gB-2 (333)  apaapaapRasggvaatvanggpasRpppvpsattKaRKRRtKKppKRp - 72
           ::::: ::::: ::::: ::::: ::::: :::::
           p
           t
gB-1 (17)  pKppRpagdnatvaaghatlRehlRdiKaentdanfyvcpptgapvvqf -127
           ::::: ::::: ::::: ::::: ::::: :::::
gB-2 (333)  peatpppdanatvaaghatlRahlRetKvenadaqfyvcpptgapvvqf -122
           ::::: ::::: ::::: ::::: ::::: :::::
-----
gC-1 (17)  mapgRvglavvlsllwlgagvsggsetastgptitagavknaseptsg - 50
           ::::: ::::: ::::: ::::: ::::: :::::
gC-2 (333)  malgRvglavglwglwlgvvvllan--aspgRtltvgpRgnasnaapsa - 48
           ::::: ::::: ::::: ::::: ::::: :::::
gC-1 (17)  spgsaaspevtptstpnnpnvtqnKttptepaspttpKotstpspspts -100
           ::::: ::::: ::::: ::::: ::::: :::::
gC-2 (333)  spRnasapRttptp-pqpRKatKsKastaKpapp----- - 82
           ::::: ::::: ::::: ::::: ::::: :::::
gC-1 (17)  tdpdKpKnnttpaKsgRptKppgpvwcdRRdplaRygsRvqiRcRfRnst -150
           ::::: ::::: ::::: ::::: ::::: :::::
gC-2 (333)  -----KtgppKtssepvRcnRhdplaRygsRvqiRcRfRnst -119
           ::::: ::::: ::::: ::::: ::::: :::::
gC-1 (17)  RmefRlqiwRysmgpsppiapadleevtnitappggllvydsapnltd -200
           ::::: ::::: ::::: ::::: ::::: :::::
gC-2 (333)  RtesRlqiwRyatatdaeigtapsleevmvnsappggqlvydsapnRtd -169
           ::::: ::::: ::::: ::::: ::::: :::::

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Fig. 5. Amino acid sequences near the N-termini of gB and gC. Numbering is from the first amino acid of the translation product including the signal sequence. For gB the first amino acid shown is the beginning of the mature protein after cleavage of the signal sequence. Alignments of the HSV-1(strain 17) and HSV-2(strain 333) forms of each protein (e.g., gB-1 and gB-2) were done using the PALIGN program in the PCGENE suite. The double dots indicate sequence identity and the single dots, sequence similarity; the dashes represent gaps introduced to maximize alignment. Analysis of these sequences and others in Version 18 of the Swiss Protein Database revealed that, in the regions shown here, gB-2 sequences for two strains were identical as were gC-1 sequences and gC-2 sequences for two strains each. gB-1 sequences for four strains revealed the sequence variations indicated by the substitutions shown above the line of strain 17 sequence. The basic amino acids are shown in underlined capital letters.

with HSV in requirements for binding to cells. For both PRV and BHV-1, (i) heparin inhibits the binding of virus to cells and infection; (ii) treatment of cells with heparitinase or heparinase destroys receptors for virus and renders the cells resistant to infection; and (iii) a viral glycoprotein homologous to HSV gC has heparin-binding activity and mediates the attachment of virus to cells (Schreurs *et al.*, 1988; Mettenleiter, 1989; Zuckermann *et al.*, 1989; Mettenleiter *et al.*, 1990; Sawitzky *et al.*, 1990; Zsak *et al.*, 1991; Okazaki *et al.*, 1991). In the case of BHV-1, the viral glycoprotein homologous to HSV gB also has heparin-binding activity (Okazaki *et al.*, 1991). Similar results were reported for PRV gII (gB) by one group (Sawitzky *et al.*, 1990) but not by another (Mettenleiter *et al.*, 1990).

Although the heparin-binding glycoproteins specified by HSV, PRV and BHV-1 have diverged considerably in amino acid sequence, common features of the proteins are discernable. For example, HSV gC and the homologous glycoproteins of PRV and BHV-1 all exhibit a very hydrophilic region, with a run of basic amino acids, near their N-termini. These herpesvirus glycoproteins provide an evolutionarily related, but divergent, set of proteins that should be very useful for studies of the specificity and physiological significance of affinity for heparin and related glycosaminoglycans.

SUMMARY

Our current incomplete picture of the earliest events in HSV infection may be summarized as follows. The initial interaction of virus with cells is the binding of virion

gC to heparan sulfate moieties of cell surface proteoglycans. Stable binding of virus to cells may require the interaction of other virion glycoproteins with other cell surface receptors as well (including the interaction of gB with heparan sulfate). Penetration of virus into the cell is mediated by fusion of the virion envelope with the cell plasma membrane. Events leading up to this fusion require the action of at least three viral glycoproteins (gB, gD and gH), one or more of which may interact with specific cell surface components. It seems likely that binding of gB to cell surface heparan sulfate may occur and may be important in the activation of some event required for virus penetration.

Heparan sulfate is present not only as a constituent of cell surface proteoglycans but also as a component of the extracellular matrix and basement membranes in organized tissues. In addition, body fluids contain both heparin and heparin-binding proteins, either of which can prevent the binding of HSV to cells (WuDunn and Spear, 1989). As a consequence, the spread of HSV infection is probably influenced, not only by immune responses to the virus, but also by the probability that virus will be entrapped or inhibited from binding to cells by extracellular forms of heparin or heparan sulfate.

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